KANEKA KanCapA™ 3G Technical Note (2)

Pressure flow characteristics & scalability of Kaneka KanCapA™ and KanCapA™ 3G

Monoclonal antibodies are highly specialized and expensive class of drugs that target various human illnesses. The higher cost of antibodies as compared to traditional small molecule drugs is partly due to the fact that higher doses (usually in order of milligrams) of antibody drugs are needed to see therapeutic effects. Consequently, large production facilities are a must to cover higher production demands. Given the recent advancements in upstream technologies leading to high titer antibody producing cell lines, it is natural that antibody manufacturing is under constant pressure to efficiently produce antibodies at production scale.

The antibody manufacturing process is first developed at small scale (milligram quantities) in R&D labs and then scaled up depending upon the process stages. Conventionally antibody purification is a multi-step process and proceeds via depth filtration of harvested cell-culture fluid (HCCF) and passing it over the protein A capture step which is the first step in a multi-step chromatographic process. Thus depending upon the volume and amount of HCCF, protein A chromatography requires the most scale up. Therefore, typical design influencers for a successful Protein A affinity chromatography scale up are high dynamic binding capacity, high flow rates, low back pressure and its ability to scale up without having significant tradeoffs between flow rates and back pressure.

In this note, we discuss the pressure flow and scalability for our KANEKA KanCapA (KanCapA) and KANEKA KanCapA 3G (KanCapA 3G) protein A affinity resins. KanCapA and KanCapA3G are both designed using cellulose base matrix with the latter having optimized protein A ligand offering high dynamic binding capacity among its other features.

Materials & Methods

KanCapA and KanCapA 3G were both packed into different internal diameter (I.D.) columns (Table1). Briefly, for KanCapA the I.D. of columns ranged from 4.4 cm to 60 cm while for KanCapA 3G we used 1 cm to 30 cm I.D. columns. KanCapA resin was packed using Flow and Axial packing methods respectively while KanCapA 3G was packed using Flow packing only.

Flow Packing

KanCapA resin was packed into Vantage VL44 (EMD Millipore), BPG 100 and BPG 300 (GE Healthcare) columns of different I.D.s (Table 1). Specifically, Vantage VL44 column was packed at 4.4 cm I.D. and 20 cm height. The GE columns i.e. BPG 100 and BPG 300 had dimensions (I.D. × height) of 10 cm × 20.1 cm and 29.6 cm × 20 cm respectively.

Prior to packing, each column and associated tubing was rinsed with water and target bed height was marked to ensure correct packing. An appropriate amount of KanCapA resin was prepared and the storage solution was changed from 20 % (v/v) Ethanol to water. The initial slurry concentration was kept at 50-60 % (w/v). Thoroughly mixed slurry was poured into column and let to settle until a clear layer could be seen at the top of the bed. Next, the top adapter was set about 1 cm above the resin bed by carefully removing any air bubbles from the filter and inlet and the pump was started at packing flow rate. Once the pressure and bed height were stabilized, the pump was stopped and bed height was marked. The top adapter was lowered to the marked position in the column and this process was repeated until the bed height (resin) did not compress anymore. After flow compression the top adapter was slowly lowered to the target bed height by axial compression manually. Once packed, the packing efficiency was checked by evaluating the plate number and asymmetric factor. In general we found that plate number to be greater than 2000 while the asymmetric factor ranged between 0.8-1.6.

KanCapA3G was packed using Tricorn 10, BPG 100 and BPG 300 at dimensions of (I.D. × height) 1 cm × 20 cm, 10 cm × 20 cm, 30 cm × 20 cm respectively by the method described above.

Axial Packing

KanCapA resin was also packed into AxiChrom 70 (GE), InPlace 446 (Bio-Rad) and AxiChrom 600 (GE) using axial packing method. Specifically the AxiChrom 70 had I.D. of 7 cm and height of 19.9 cm; AxiChrom 600 had I.D. of 60 cm and height of 25 cm while InPlace 446 column had dimensions of 44.6 cm I.D. and 23.3 cm height. Each column was filled with water to purge the air from column and all the lines. Next, an appropriate amount of KanCapA resin was prepared and the storage solution was changed from 20% (v/v) ethanol to water. The initial slurry concentration was kept at 50-60 %(w/v). Thoroughly mixed slurry was poured into the column and the adapter was allowed to move at a fixed speed (From 20 cm/h to 100 cm/h). Once the adapter reached the marked target height, the system was stopped and the properties of packed columns were evaluated by measuring the plate number and packing asymmetry. As noted previously the plate number was found to be greater than 2000, and asymmetric factor was in range of 0.8-1.6.

Table 1. Details of packing

Resin	Column (manufacturer)	Equipment	Packing method	I.D. (mm)	Height (mm)	CF	Plates/m	Asymmetry
KanCapA	VantageL44 (Millipore)	AKTA pilot	Flow packing	44	200	110	5710	1.14
	AxiChrom 70 (GE healthcare)	AKTA pilot	Axial compression	70	199	110	6063	1.06
	BPG 100 (GE healthcare)	AKTA process	Flow packing	100	201	110	5276	1.18
	BPG 300 (GE healthcare)	LabTOP 300	Flow packing	296	200	110	5208	1.17
	InPlace 446 (BIO-RAD)	LabTop 400	Axial compression	446	233	110	4712	1.09
	AxiChrom 600 (GE healthcare)	AKTA process	Axial compression using AxiChrom Master	600	250	110	6257	1.06
KanCapA 3G	Tricorn 10 (GE healthcare)	Akta Avant 150	Flow packing	10	200	100	3153	1.35
	BPG 100 (GE healthcare)	AKTA process	Flow packing	100	200	110	5475	1.15
	BPG 300 (GE healthcare)	LabTOP 300	Flow packing	300	200	110	3732	1.15

Results & Discussions

Measurement of Pressure flow properties

We explored the pressure flow properties of the packed bed with water flow up to 700 cm/h. We evaluated the relationship between pressure and linear flow rate for KanCapA resin packed by flow packing in 4.4 cm (I.D.) × 20 cm (height) dimension column. Our data (Fig. 1) shows a linear relationship between pressure and flow rate (up to 800 cm/h). Specifically at 700 cm/h, we found the pressure to be at 0.22 MPa.

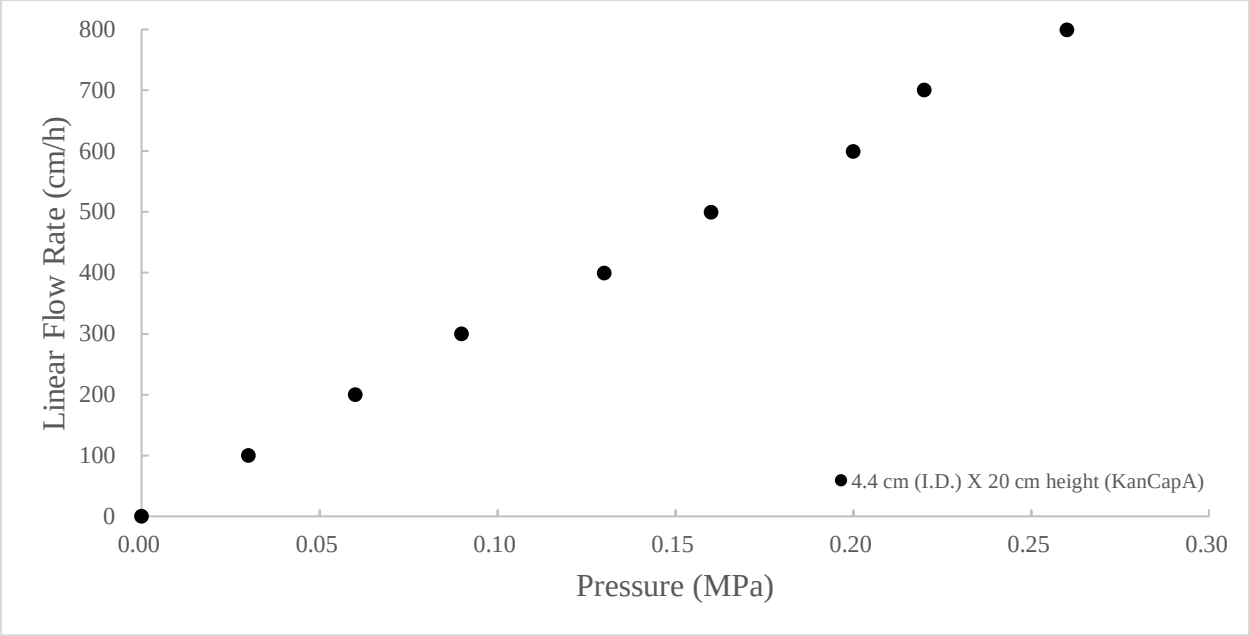

Figure 1. Pressure vs. Linear flow rate for KANEKA KanCapA™ in 4.4 cm I.D. column

To demonstrate scalability of KanCapA, we further evaluated the pressure and flow rate properties for a 10 cm (I.D.) × 20 cm (height) and 30 cm (I.D.) × 20 cm (height) respectively. To maintain consistency, we packed both of these columns using flow packing as well. As shown in figure 2, the pressure-flow rate trends are similar to as observed for 4.4 cm (I.D.) column suggesting good scalability of KanCapA. Typical compression factors for all these three cases were 110%.

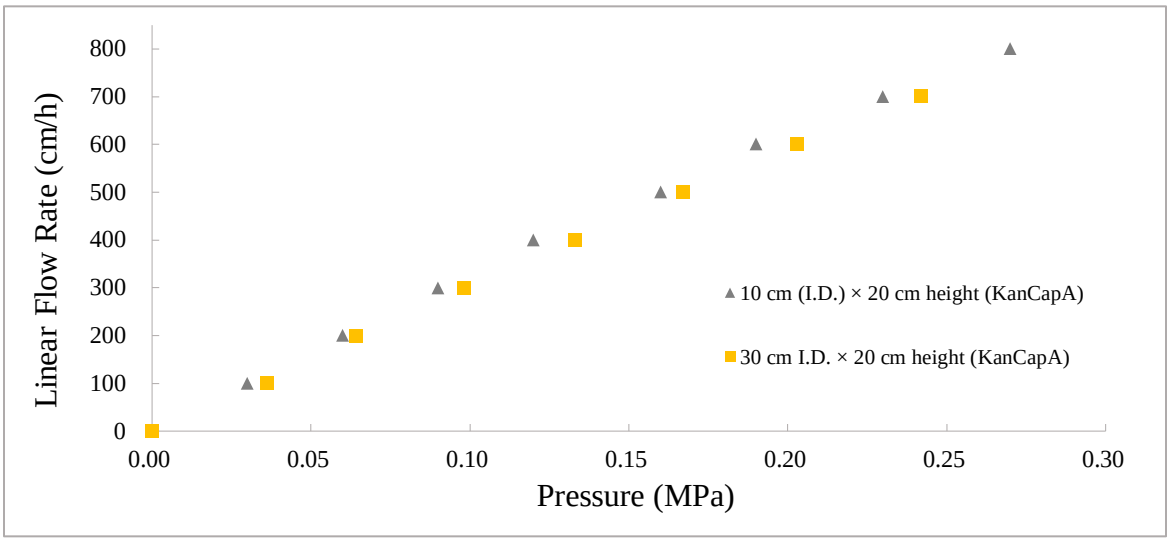

Figure 2. Pressure vs. Linear flow rate for KANEKA KanCapA™ in 10 cm I.D. and 30 cm I.D. columns respectively

Next, we evaluated the pressure flow-rate relationship for columns packed using axial compression. We first used a column of 7.0 cm (I.D.) × 20 cm (height) and note that up to 700 cm/h of flow rate the relationship is linear (Fig. 3), akin to the one observed for the KanCapA packed using flow packing.

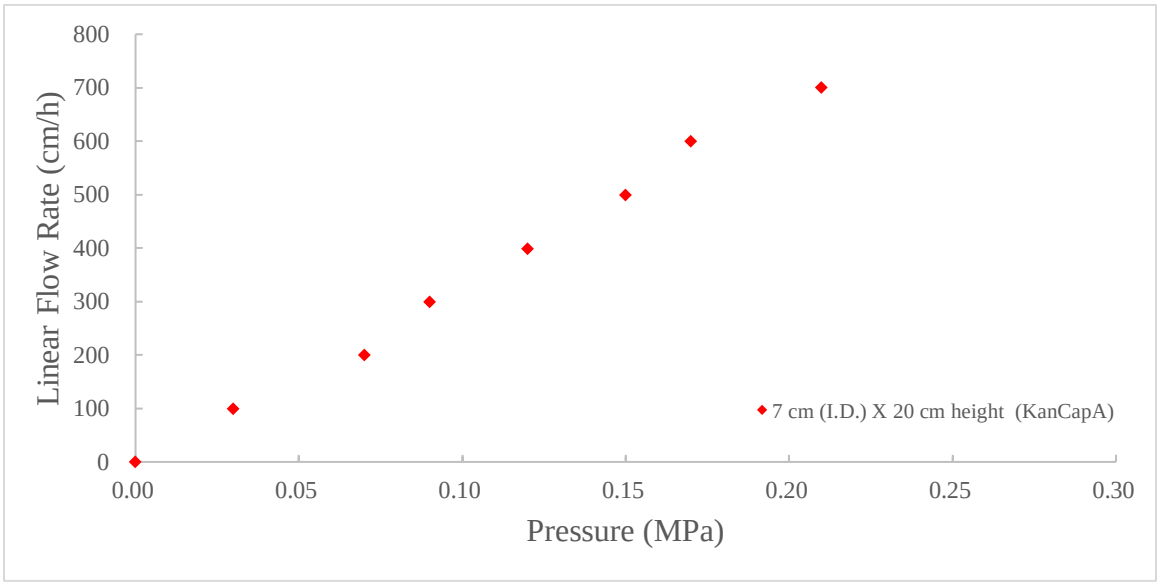

Figure 3. Pressure vs. Linear flow rate for KANEKA KanCapA™ in 7 cm I.D. packed using Axial

compression method

To emulate process scale up, we next evaluated columns of 45 cm (I.D.) × 23 cm (height) and; 60 cm (I.D.) × 25 cm (height) dimensions respectively packed using axial compression method. Interestingly our data showed excellent linearity between pressure and flow rates for these dimensions suggesting excellent scalability of KanCapA resin (Fig. 4).

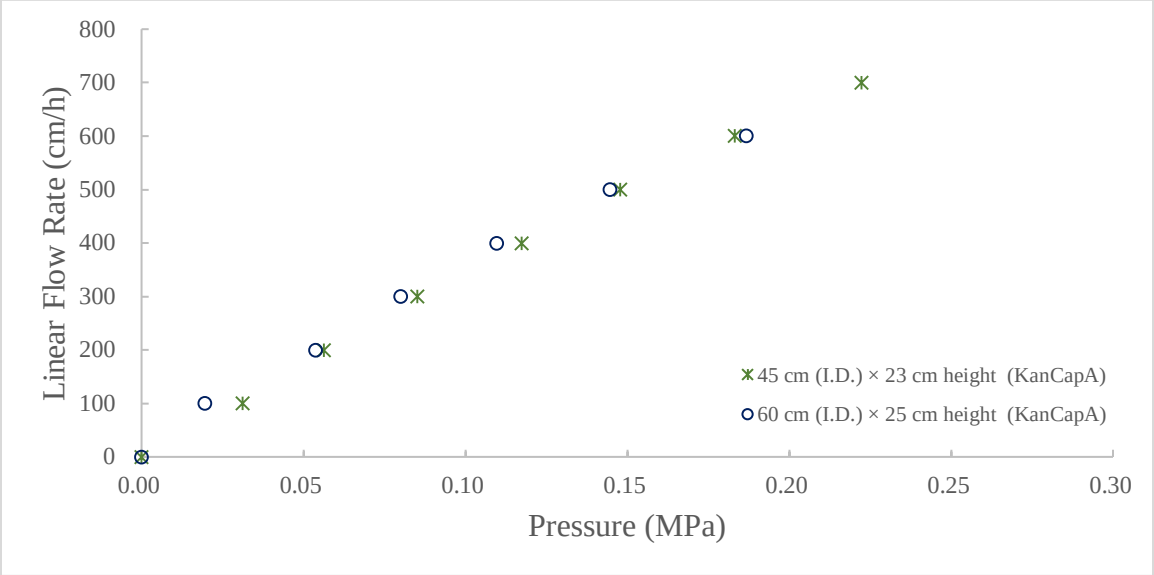

Figure 4. Pressure vs. Linear flow rate for KANEKA KanCapA™ in 45 cm I.D. and 60 cm I.D. columns respectively. Both columns were packed using Axial compression method

We tested 1.0 cm (I.D.), 10 cm (I.D.) and 30 cm (I.D.) columns with a height of 20 cm for KanCapA3G using flow packing method. Our data showed similar linear relationship between pressure and flow rate (Fig. 5).

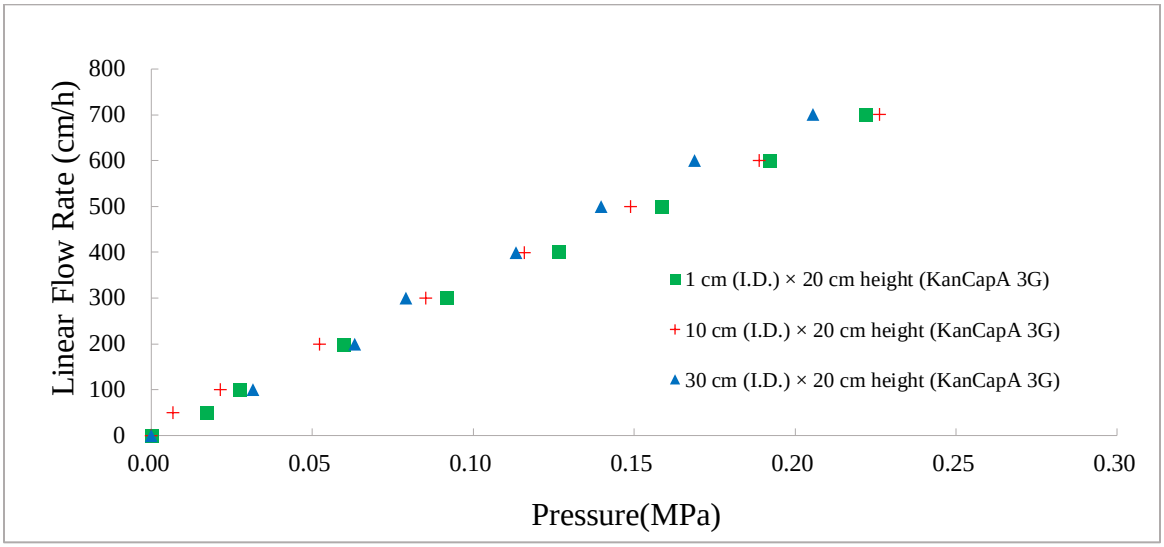

Figure 5. Pressure vs. Linear flow rate for KANEKA KanCapA™ 3G resin packed using flow method

Upon comparison of the pressure flow and linear flow rate data for 30 cm (I.D.) columns for KanCapA and KanCapA3G (both packed using flow packing), we observed no significant variations in the relationship between the two resins (Fig. 6).

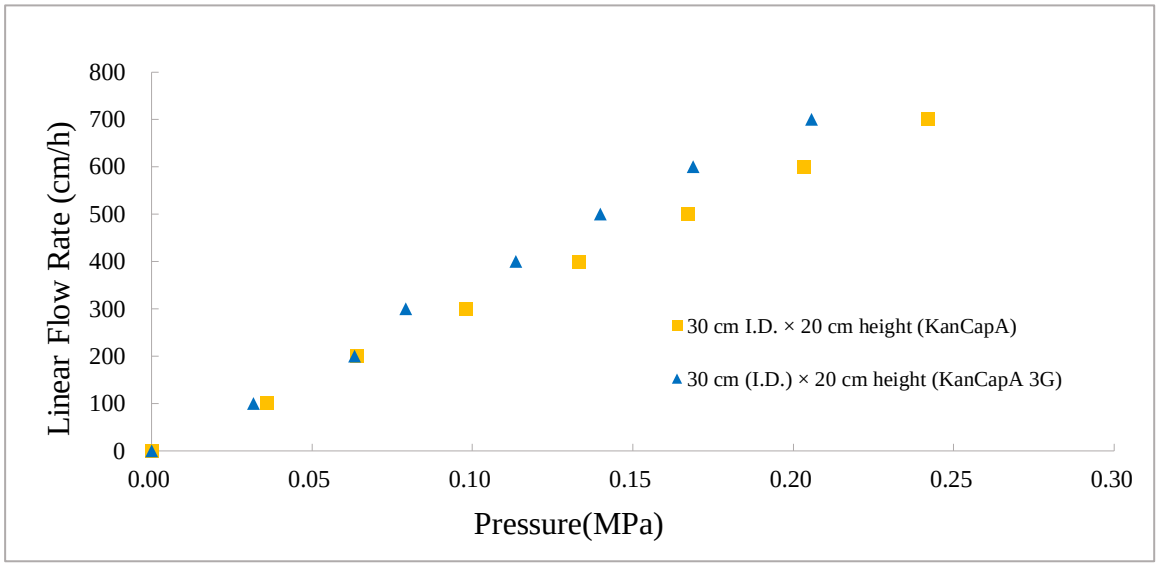

Figure 6. Comparison of pressure vs. linear flow rate for KANEKA KanCapA™ and KANEKA KanCap™ 3G for 30 cm (I.D.) × 20 cm (height) showing similar properties

Furthermore, as noted above that KanCapA and KanCapA 3G share the same base matrix, it is relatively safe to assume that KanCapA 3G can also be packed into larger dimension (process scale) columns without any significant trade-offs between pressure and flow rate. As shown in Figure 7, there is excellent linearity between pressure and flow rate for columns of various dimensions packed with either flow or axial packing using KanCapA or KanCapA3G suggesting robust resins amenable to scalability. Finally, for all the columns packed in this note, the compression factors for KanCapA resin were 110 % and ranged from 100-110% for KanCapA 3G whereas the plate number for each resin packed was >2000 with asymmetry ranging from 1.06-1.35 (Table 1).

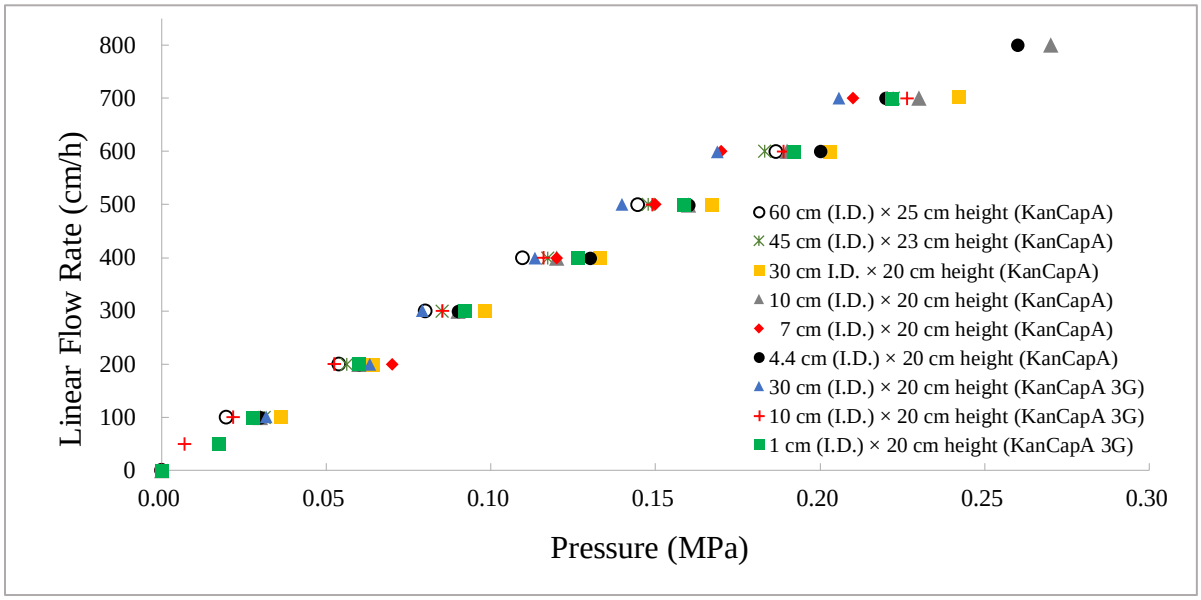

Figure 7. Comparison of pressure vs. linear flow rate for KANEKA KanCapA™ and KanCapA3G™ resins

Conclusions

With the rising demands of antibody drugs and increasing calls for manufacturers to speed up the manufacturing process and lower the manufacturing costs, it is imperative that scale up process be straightforward and as painless as possible. Our KanCapA and KanCapA 3G resins exhibit excellent

packing (with different methods) and scalability properties without any significant trade-offs. However, we realize that antibody manufacturing may require even further larger dimension columns. Therefore we went a step ahead and evaluated the packing ability of our base matrix. We packed our base matrix into Pall Biotech's Resolute® column with dimensions of 140 cm (I.D.) and 20 cm (height). We prepared a 65% (w/v) slurry concentration using water and packed using axial compression method. As noted above that a well packed column should exhibit plate number >2000 with asymmetry to be between 0.8-1.6. Our data for KanCapA series base matrix shows the plate numbers to be 6511.7 ± 258.9 while the asymmetry was estimated to be 1.09 ± 0.09. Interestingly the compression factor for base matrix packing was 106.66 ± 3.51, in line with reported values for KanCapA and KanCapA3G resins as shown in Table 1. This data clearly shows that the base matrix can be packed into a 140 cm I.D. column without any issues. Since KanCapA and KanCapA 3G both share the same cellulose base matrix, it is thus logical to conclude that these two resins should be scalable to large columns suitable for manufacturing scale and thus are poised to address challenges associated with large scale manufacturing of monoclonal antibodies without much bargain between pressure and flow rates.

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